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Influence of inflammatory bowel disease on intestinal microflora

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SUMMARY The microflora of the jejunum, ileum, and colon has been studied from operative samples in Crohn's disease (n = 30), ulcerative colitis (n = 15), and controls (n = 40). There was no significant difference in the flora of patients with ulcerative colitis compared with controls. In Crohn's disease there was a significant increase in *E. coli* ($P < 0.001$) and *B. fragilis* ($P < 0.001$) in the ileum and of *E. coli* ($P < 0.001$) and *lactobacilli* ($P < 0.01$) in the colon. The abnormal ileal flora in Crohn's disease was unrelated to serological evidence of disease activity (indices: ESR, serum albumin, serum seromucoids), diameter of the ileum, or excision of the ileocaecal valve. The abnormal colonic flora in Crohn's disease was not related to presence of macroscopic colitis.

An abnormal small intestine microflora has been demonstrated in some patients with Crohn's disease (Drasar *et al.*, 1969; Gorbach and Tabaqchali, 1969; Gorbach, 1971). There is however, little information on the microflora of the large intestine in inflammatory bowel disease. Such evidence as does exist suggests that, in ulcerative colitis, the small and large intestinal microflora does not differ significantly from that in normal subjects (Jacomina *et al.*, 1975). It has been suggested that the abnormal intestinal flora in Crohn's disease may be the main reason for a high incidence of sepsis after intestinal resection (Barker *et al.*, 1971; Sykes, 1975).

This study has examined the aerobic and anaerobic flora of the small and large bowel in patients with inflammatory bowel disease compared with normal subjects.

Methods

PATIENTS

We have studied 45 consecutive patients undergoing elective bowel resection for Crohn's disease (n = 30) or ulcerative colitis (n = 15) in the 12 months from July 1976 to June 1977. Results in patients with inflammatory bowel disease have been compared with those from 40 controls. The controls included 29 patients undergoing elective peptic ulcer surgery, staging laparotomy, or cholecystectomy and 11 patients with haemorrhoids referred to our rectal clinic for sigmoidoscopy. In the operated patients fluid samples were aspirated from the jejunum, ileum, and transverse colon using the dilution technique of Nichols *et al.* (1972). In the non-operated patients stool samples were used for analysis. Crohn's patients and controls were excluded if operative cultures from the bile or gastric aspirates were infected because bile and gastric contents are usually sterile (Csendes *et al.*, 1975; Gatehouse *et al.*, 1978) as overgrowth in the stomach or biliary tract is known to have a profound influence on small intestinal microflora (Gorbach, 1971). None of the patients had received any form of mechanical bowel preparation, preoperative oral antimicrobials, or prophylactic systemic antibiotic cover.

Details of the patients with inflammatory bowel disease are listed in Table 1. There were 31 operations on 30 patients with Crohn's disease and 16 operations for the 15 patients with ulcerative colitis.

We have also studied the microflora of ileostomy patients by intubating the stoma of 12 healthy volunteers attending a stomatherapy clinic who had had a previous proctocolectomy for ulcerative colitis. This was necessary in order to compare ileal fluid in controls with ileal contents of patients with an ileostomy.

Techniques

Operative samples of intestinal fluid were obtained using a modification of the technique described by Nichols *et al.* (1972). Ten millilitres of isotonic

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Table 1 *Patients studied*

	Crohn's disease	*Ulcerative colitis*
Number of patients (No. of operations)	30(31)	15(16)
Previous operation		
None	13	10
Total colectomy		
With anastomosis	1	2
With ileostomy	3	4
Ileostomy alone	2	0
Excision of ileocaecal valve	12	0
Site of disease		
Small bowel alone	20	—
Ileum and colon	6	—
Colon alone	5	16
Operations		
Small bowel resection alone	17	—
with caecum	6	—
Total colectomy with anastomosis	3	0
with ileostomy	2	7
Rectal excision alone	1	4
Re-fashioning ileostomy	0	2
Others		
Examination under anaesthesia, proximal gastric vagotomy, cholecystomy	2	3

saline were injected into a 10 cm segment of bowel isolated between non-crushing clamps. After thorough mixing for one minute the fluid was aspirated and taken immediately to the microbiology laboratory. Samples were collected, where possible, from the jejunum (10 cm from the duodenojejunal flexure), the terminal ileum (10 cm from the ileocaecal valve or previous suture line) and the colon (usually transverse colon). In some cases stool samples were used for analysis where collection from the transverse colon had not been technically feasible. Solid stool specimens collected during sigmoidoscopy were homogenised with 10 ml of saline, whereas operative colonic samples were always fluid after injecting 10 ml of saline into the isolated segment of the colon. All results were, therefore, expressed as number of organisms per ml. This study is concerned with the concentration of bacteria in the bowel rather than their absolute numbers, so that recovery of all the diluent injected into the bowel at operation was not insisted upon (Bentley *et al.*, 1972).

Serial dilutions of intestinal contents were prepared within 20 minutes of collection in an anaerobic cabinet on seven different pre-reduced selective media using the single drop technique of Miles and Misra (1938). Plates were incubated aerobically and anaerobically at 37°C and read at 72 hours.

The extent of abnormal microflora in the small bowel was compared with (1) the state of disease activity as measured by albumin, ESR, and seromucoid (Cooke, *et al.*, 1958); (2) the presence of enteroenteric fistulae confirmed at operation; (3) the degree of stenosis measured in the unfixed specimen by graduated metal sounds of known diameter; and (4) previous excision of the ileocaecal value.

As logarithmic counts of viable organisms conformed to that of a normal distribution curve we have used Student's *t* test for statistical analysis of the logarithmic data.

Results

JEJUNAL COUNTS

The mean jejunal counts of the 14 most frequently isolated intestinal bacteria in patients with Crohn's disease (n = 23) and ulcerative colitis (n = 10) have been compared with those of controls (n = 27) (Table 2). There was no significant difference between the bacterial flora of the jejunum in the three groups. Nevertheless, there was a small number of patients with Crohn's disease who had extremely high counts of *E. coli* and *B. fragilis* compared with controls (Figs. 1 and 2).

ILEAL COUNTS

There was no difference between the ileal flora of patients with ulcerative colitis (n = 11) when compared with controls (n = 28) (Table 3). However, in Crohn's disease (n = 26) there was a significant increase in the number of *E. coli* (4×10^5) compared with controls (9×10^1: $P < 0.001$) and of *B. fragilis* (6×10^5) when compared with controls (4×10^1: $P < 0.001$). There was no correlation between the height of the ESR and the ileal counts of *E. coli* ($r = 0.04$) or *B. fragilis* ($r = -0.12$). No correlation was found between serum seromucoid and the numbers of *E. coli* in the ileum ($r = -0.05$) or the numbers of *B. fragilis* in the ileum ($r = -0.08$). There was no correlation

Table 2 *Mean counts per ml of organisms in jejunum (logarithmic values)*

	Controls n = 27	*Crohn's disease n = 23*	*Ulcerative colitis n = 10*
Escherichia coli	0·99 ± 0·81	1·46 ± 2·01	0·72 ± 0·60
Streptococcus faecalis	1·35 ± 1·12	1·03 ± 1·27	0·83 ± 0·83
Klebsiella aerogenes	0	1·41 ± 2·36	0
Proteus species	0	0	0·91 ± 1·13
Streptococcus viridans	1·75 ± 1·23	1·78 ± 0·92	0·70 ± 0·57
Staphylococcus albus	0·74 ± 0·62	0·80 ± 1·02	0·67 ± 0·61
Aerobic lactobacilli	0·72 ± 0·63	1·39 ± 1·76	0
Bacteroides fragilis	0·62 ± 0·52	1·42 ± 2·17	0
Bacteroides melaninogenicus	0	0	0
Bifidobacterium species	0	0·87 ± 1·30	0
Clostridium welchii	0·67 ± 0·61	1·02 ± 1·37	0
Anaerobic streptococcus	0	0·93 ± 1·12	0
Veillonella species	0	0·67 ± 0·53	0
Yeasts	0·83 ± 0·81	0·93 ± 0·69	0·80 ± 0·89

± 1 SD.

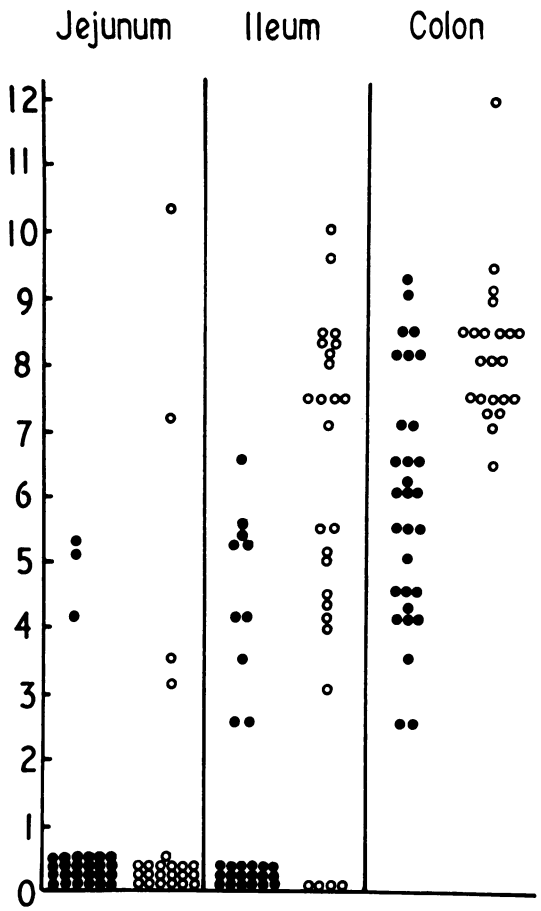

Fig. 1 *Viable counts of* E. coli *(logarithmic scale).* ●*Control.* ○*Crohn's disease.*

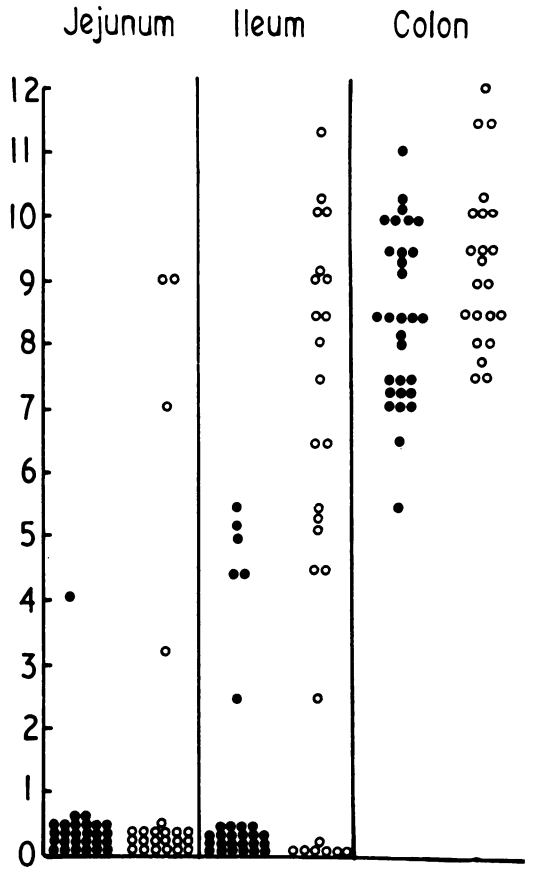

Fig. 2 *Viable counts of* B. fragilis *(logarithmic scale).* ●*Control.* ○*Crohn's disease.*

between serum albumin levels and the ileal counts of *E. coli* ($r = 0{\cdot}09$) or *B. fragilis* ($r = 0{\cdot}31$). Presence of an enteroenteric fistula, an ileal diameter of less than 15 mm, or previous ileocaecectomy was associated with only a very small increase in the numbers of *E. coli* and *B. fragilis* (Table 4), and these differences were not statistically significant.

Because the presence of an ileostomy might influence the intestinal flora of the ileum (Finegold *et al.*, 1970) and as nine of the 45 patients with inflammatory bowel disease had been treated previously by ileostomy, we have compared ileostomy flora with the ileal flora of controls. Intubation of the ileostomy provided samples where the mean *E. coli* count was 9×10^2 and the mean counts of *B. fragilis* were 9×10^1. These results did not differ significantly from our controls.

COLONIC COUNTS

Analysis of the numbers of organisms isolated from the colon (Table 5) has shown no significant difference between patients with ulcerative colitis ($n = 12$) and controls ($n = 30$). In Crohn's disease ($n = 22$) there was a significant increase in numbers of *E. coli* (2×10^8) compared with controls (8×10^5: $P < 0{\cdot}001$) and of *lactobacilli* (8×10^4) compared with controls (2×10^2: $P < 0{\cdot}01$). There was also an increase in the numbers of *B. fragilis* and *veillonella* in the colon in Crohn's disease as compared with the controls, but the difference was not statistically significant. There was no significant difference in the counts of *E. coli* and *lactobacilli* according to whether or not there was radiological evidence of Crohn's colitis (Table 6).

The operative flora from the large bowel have been compared with those from sigmoidoscopy specimens in controls. The mean *E. coli* counts obtained by needle aspiration of the transverse

Table 3 *Mean counts per ml of organisms in the ileum (logarithmic values)*

	Controls n = 28	*Crohn's disease n = 26*	*Ulcerative colitis n = 11*
Escherichia coli	1·92 ± 1·65	5·39 ± 2·39*	1·41 ± 1·63
Streptococcus faecalis	2·51 ± 1·59	2·93 ± 2·51	1·72 ± 1·31
Klebsiella aerogenes	0·89 ± 0·86	1·16 ± 1·47	1·42 ± 1·79
Proteus species	0	1·37 ± 1·57	0
Streptococcus viridans	1·67 ± 1·34	1·96 ± 1·67	1·43 ± 1·35
Staphylococcus albus	0·89 ± 0·81	0·82 ± 0·89	2·21 ± 2·72
Aerobic lactobacilli	1·62 ± 1·43	3·19 ± 2·71	1·91 ± 1·53
Bacteroides fragilis	1·37 ± 1·29	5·65 ± 3·23*	1·54 ± 1·70
Bacteroides melaninogenicus	0·96 ± 1·20	0	0
Bifidobacterium species	0·83 ± 1·14	1·19 ± 1·42	0
Clostridium welchii	1·83 ± 1·51	2·39 ± 1·87	1·32 ± 1·12
Anaerobic streptococcus	0·73 ± 0·72	1·52 ± 2·02	0
Veillonella species	0·69 ± 0·71	1·37 ± 1·72	0
Yeasts	0·82 ± 0·62	0·91 ± 0·72	0·71 ± 0·72

*P < 0·001.

Table 5 *Mean counts per ml of organisms in colon (logarithmic values)*

	Controls n = 30	*Crohn's disease n = 22*	*Ulcerative colitis n = 12*
Escherichia coli	5·78 ± 2·10	8·22 ± 1·93*	6·94 ± 1·34
Streptococcus faecalis	3·72 ± 2·91	4·60 ± 2·31	4·92 ± 1·99
Klebsiella aerogenes	1·39 ± 1·51	0·84 ± 0·91	2·22 ± 2·92
Proteus species	1·57 ± 1·63	2·87 ± 2·36	1·32 ± 1·36
Streptococcus viridans	2·19 ± 2·23	2·79 ± 2·34	2·82 ± 2·19
Staphylococcus albus	1·71 ± 1·83	1·31 ± 0·92	0
Aerobic lactobacilli	2·16 ± 2·31	4·86 ± 2·94†	3·97 ± 2·91
Bacteroides fragilis	8·31 ± 2·12	9·12 ± 1·37	7·85 ± 1·93
Bacteroides melaninogenicus	0·09 ± 1·21	0	1·79 ± 1·92
Bifidobacterium species	2·01 ± 2·37	3·01 ± 2·57	1·42 ± 1·73
Clostridium welchii	3·69 ± 2·91	3·75 ± 2·71	2·95 ± 2·54
Anaerobic streptococcus	1·31 ± 1·71	3·32 ± 3·30	2·34 ± 2·81
Veillonella species	2·19 ± 2·63	3·83 ± 3·71	1·12 ± 0·32
Yeasts	0·72 ± 0·61	0·72 ± 0·83	0

*P < 0·001.
†P < 0·01.

colon (7×10^5) were similar to those obtained by sigmoidoscopy (6×10^6). Furthermore, the counts of *B. fragilis* from the transverse colon (1×10^8) did not differ from those from stool specimens (2×10^8).

Because samples were sometimes obtained from the right or left colon rather than transverse colon, multiple colonic samples (right colon, transverse and sigmoid colon) were examined from eight patients with inflammatory bowel disease. Results have shown that there was no significant difference in the counts of *E. coli*, *B. fragilis*, *Streptococcus faecalis*, or *lactobacilli* from the three sites (Table 7).

Discussion

We believe that the operative method used to sample small and large bowel flora is more acceptable to the patient than per oral intubation, particularly in patients who have already been extensively investigated. The technique of operative needle aspiration is less likely to be influenced by contamination from oropharyngeal micro-organisms than the intubation methods used by most earlier investigators (Shiner, 1963; Donaldson, 1964).

We considered the possibility that needle aspiration of the colon might be responsible for an increased risk of sepsis. Despite the evidence of Nichols and others (1972) of the safety of the technique, we were concerned lest needle aspiration might increase the risk of wound infection, particularly in the control patients. We therefore monitored each control case and analysed the results sequentially to satisfy ourselves that there was no excess risk. Wound sepsis was recorded in four of the 29 controls (14%), which is similar to our rate of wound infection in elective peptic ulcer surgery (15%—Gatehouse *et al.*, 1977) and for biliary operations (18%—Keighley, 1977).

The patients underwent surgery for a wide variety of lesions but these have not been analysed separately. Many of them had undergone previous excision of the ileocaecal valve which might allow abnormal reflux of colonic bacteria into ileum. However, the data in Table 4 show that previous resection of the ileocaecal valve was not associated with a significant increase in the flora of the small bowel in Crohn's disease.

None of the patients in the study had received preoperative antibiotics or sulphasalazine in the two weeks before operation. Neither the patients nor controls received any form of mechanical bowel preparation. All subjects were given a single dose of lincomycin and gentamicin during the operation immediately after the samples for flora studies had been collected, so that they were protected against the risk of postoperative sepsis (Stokes *et al.*, 1974).

Table 4 *Mean counts per ml of* E. coli *and* Bacteroides fragilis *in ileum in Crohn's disease (logarithmic values)*

	E. coli	*B. fragilis*		*E. coli*	*B. fragilis*
Enteroenteric fistulae	7·16 ± 2·36	7·17 ± 2·79	No fistulae	5·26 ± 2·72	5·18 ± 3·41
Ileal lumen < 15 mm	6·67 ± 2·73	6·27 ± 3·31	Lumen > 15 mm	4·83 ± 2·59	4·85 ± 3·29
Previous ileocaecal resection	6·88 ± 2·17	4·88 ± 2·89	No previous resection	6·52 ± 2·91	4·82 ± 3·70
			NS		

Table 6 *Mean counts per ml of* E. coli *and* lactobacilli *in colon in Crohn's disease (logarithmic values)*

	E. coli	*Lactobacilli*		*E. coli*	*Lactobacilli*
Colonic disease	8·69 ± 1·14	5·19 ± 2·31	No colonic disease	8·01 ± 1·37	4·83 ± 2·97
			NS		

None of the patients developed pseudomembranous colitis, even though lincomycin and gentamicin were used for prophylaxis.

There was no standardisation of diet before operation in either the patients or controls. There is evidence to suggest that diet may be related to changes in the bacterial flora of the small and large bowel (Gorbach *et al.*, 1967) but the daily observed variations in faecal flora are so great that the small differences due to diet are unlikely to explain the changes observed in this study (Sanborn, 1931).

The results of this study indicate that there are profound changes in the microflora of the small and large bowel in some patients with Crohn's disease. These changes have not been observed in patients with ulcerative colitis. The significance of the increased numbers of *lactobacilli* from the colon is uncertain for similar counts were found in ulcerative colitis and in a previously studied group of patients with large bowel cancer (Arabi *et al.*, 1978). It is possible that these findings were due to an unusually low incidence of colonic *lactobacilli* in the controls. Furthermore a larger sample might demonstrate significantly increased numbers of *B. fragilis* and *veillonella* in the colon of patients with Crohn's disease.

We were surprised to find that the abnormal small bowel microflora in Crohn's disease was unrelated to biochemical evidence of active Crohn's disease. It could be argued that a clinical disease activity index using criteria such as pain, diarrhoea, and malaise might be more appropriate for correlation with our microbiological results (Best *et al.*, 1976). Unfortunately, the clinical variables used to define active disease are non-specific and could be caused by entirely separate pathological processes. Although haemoglobin and sedimentation rate are unreliable indices for activity in Crohn's disease, reduction in serum albumin and a rise in serum seromucoid have been shown to be satisfactory indicators of active Crohn's disease (Cooke *et al.*, 1958). Our results have also shown that overgrowth of intestinal bacteria in Crohn's disease is unrelated to diameter of the small bowel or surgical removal of the ileocaecal valve. It has been suggested that despite the absence of fibrous strictures, Crohn's disease may cause functional stasis which is responsible for the abnormal ileal flora in small intestinal Crohn's disease (Drasar *et al.*, 1969; Sykes *et al.*, 1975). It may be argued that anaesthetic agents might induce changes in motility of the bowel causing transient overgrowth of bacteria. The anaesthetic drugs used in patients with Crohn's disease did not differ from those used in the control group. Furthermore, there was no difference in bacterial counts between samples obtained either at the beginning or near the end of the operations. Pharmacological data do not support the hypothesis that changes in motility give rise to overgrowth of intestinal microflora (Summers and Kent, 1970).

There are two important implications of these findings. They could explain the reason for evidence of the blind loop syndrome and steatorrhoea in some patients with small bowel Crohn's disease (Gorbach and Tabaqchali, 1969). However, the most important implication of these findings is that certain patients with Crohn's disease requiring small bowel resection have bacterial counts which are similar to the bacterial counts normally found in the colon. These patients are therefore at risk from developing postoperative infections due to endogenous intestinal bacteria disseminated at operation and may constitute a group in whom antibiotic prophylaxis should be advised (Keighley, 1977).

Table 7 *Mean counts of* E. coli, lactobacilli, Streptococcus faecalis *and* B. fragilis *in eight patients where samples have been obtained from different sites in the colon (logarithmic values)*

	Caecum $n = 8$	*Transverse* $n = 8$	*Sigmoid* $n = 8$
E.coli	6·79 ± 2·34	5·63 ± 2·57	5·69 ± 2·34
Lactobacilli	4·24 ± 2·97	4·22 ± 2·90	4·46 ± 3·04
Streptococcus faecalis	3·87 ± 1·74	3·74 ± 1·63	3·59 ± 1·39
B. fragilis	8·84 ± 1·62	8·75 ± 1·14	8·80 ± 1·32
NS			

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